

DIVER1510WO-1.ST25.txt  
SEQUENCE LISTING

<110> DIVERSA CORPORATION

SHORT, Jay

<120> WHOLE CELL ENGINEERING BY MUTAGENIZING A SUBSTANTIAL PORTION OF A STARTING  
GENOME, COMBINING MUTATIONS, AND OPTIONALLY REPEATING

<130> DIVER1510WO-1

<140> PCT/US 01/19367

<141> 2001-06-14

<150> US 09/677,584

<151> 2000-09-30

<150> US 09/594,459

<151> 2000-06-14

<160> 33

<170> PatentIn version 3.0

<210> 1

<211> 5818

<212> DNA

<213> Escherichia coli

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<400> 2

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 35 40 45  
 Ser Ser Glu Gly Ser Glu Ala Met Gln Glu Gly Ala Tyr Arg Phe Tyr  
 50 55 60  
 Arg Asn Pro Asn Val Ser Ala Glu Ala Ile Arg Lys Ala Gly Ala Met  
 65 70 75 80  
 Gln Thr Val Lys Leu Ala Gln Glu Phe Pro Glu Leu Leu Ala Ile Glu  
 85 90 95  
 Asp Thr Thr Ser Leu Ser Tyr Arg His Gln Val Ala Glu Glu Leu Gly  
 100 105 110  
 Lys Leu Gly Ser Ile Gln Asp Lys Ser Arg Gly Trp Trp Val His Ser  
 115 120 125

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Val Leu Leu Glu Ala Thr Thr Phe Arg Thr Val Gly Leu Leu His  
130 135 140  
Gln Glu Trp Trp Met Arg Pro Asp Asp Pro Ala Asp Ala Asp Glu Lys  
145 150 155 160  
Glu Ser Gly Lys Trp Leu Ala Ala Ala Ala Thr Ser Arg Leu Arg Met  
165 170 175  
Gly Ser Met Met Ser Asn Val Ile Ala Val Cys Asp Arg Glu Ala Asp  
180 185 190  
Ile His Ala Tyr Leu Gln Asp Arg Leu Ala His Asn Glu Arg Phe Val  
195 200 205  
Val Arg Ser Lys His Pro Arg Lys Asp Val Glu Ser Gly Leu Tyr Leu  
210 215 220  
Ile Asp His Leu Lys Asn Gln Pro Glu Leu Gly Gly Tyr Gln Ile Ser  
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260 265 270  
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305 310 315 320  
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325 330 335  
Gly Ala Glu Arg Gln Arg Met Glu Glu Pro Asp Asn Leu Glu Arg Met  
340 345 350  
Val Ser Ile Leu Ser Phe Val Ala Val Arg Leu Leu Gln Leu Arg Glu  
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Ser Phe Thr Leu Pro Gln Ala Leu Arg Ala Gln Gly Leu Leu Lys Glu  
370 375 380  
Ala Glu His Val Glu Ser Gln Ser Ala Glu Thr Val Leu Thr Pro Asp  
385 390 395 400  
Glu Cys Gln Leu Leu Gly Tyr Leu Asp Lys Gly Lys Arg Lys Arg Lys  
405 410 415  
Glu Lys Ala Gly Ser Leu Gln Trp Ala Tyr Met Ala Ile Ala Arg Leu  
420 425 430  
Gly Gly Phe Met Asp Ser Lys Arg Thr Gly Ile Ala Ser Trp Gly Ala  
435 440 445  
Leu Trp Glu Gly Trp Glu Ala Leu Gln Ser Lys Leu Asp Gly Phe Leu  
450 455 460

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Ala Ala Lys Asp Leu Met Ala Gln Gly Ile Lys Ile  
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<210> 3  
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<212> DNA  
<213> Artificial sequence

<220>  
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<222> (1)..(30)  
<223> n is A, T, G, or C

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30

<210> 4  
<211> 30  
<212> DNA  
<213> Artificial sequence

<220>  
<223> Defined sequence kernel

<220>  
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<222> (1)..(30)  
<223> n is A, T, G, or C

<400> 4  
nnnnnnnnnn nnnnnnnnnn mnnnnnnnnn

30

<210> 5  
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<220>  
<223> Antibody spacer peptide. The entire peptide sequence can be repeated more than one time

<400> 5

Gly Gly Gly Gly Ser  
1 5

<210> 6  
<211> 14  
<212> DNA  
<213> Artificial sequence

<220>  
<223> Tetradecanucleotide d

<400> 6  
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14

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<210> 7  
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 <212> DNA  
 <213> Artificial sequence

<220>  
 <223> 21-mer d

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21

<210> 8  
 <211> 12  
 <212> DNA  
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<220>  
 <223> 12-mer target DNA

<400> 8  
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12

<210> 9  
 <211> 12  
 <212> DNA  
 <213> Artificial sequence

<220>  
 <223> Complement of the original 12-mer target

<400> 9  
 tcggatcgac tt

12

<210> 10  
 <211> 4  
 <212> PRT  
 <213> Artificial sequence

<220>  
 <223> Target sequence

<220>  
 <221> VARIANT  
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 <223> Xaa is any Amino Acid

<400> 10

Tyr Tyr Xaa Tyr  
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<210> 11  
 <211> 4  
 <212> PRT  
 <213> Artificial sequence

<220>  
 <223> Single base mismatched probe

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Tyr Tyr Tyr Tyr  
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<210> 12

<211> 4

<212> PRT

<213> Artificial sequence

<220>

<223> 4-mer extemtion probe

<220>

<221> VARIANT

<222> (2)..(2)

<223> Xaa is any Amino Acid

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Tyr Xaa Tyr Tyr  
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<210> 13

<211> 10

<212> DNA

<213> Artificial sequence

<220>

<223> BstNB I cleaves btw. nucleotide 9 & 10 of target sequence

<220>

<221> misc\_feature

<222> (6)..(10)

<223> n is any nucleotide

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10

<210> 14

<211> 223

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<223> Forward primer

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<221> misc\_feature

<222> (1)..(223)

<223> n is any nucleotide

<220>

<221> misc\_feature

<222> (1)..(10)

<223> at least one nt. of nt. 1-10 is present

<220>

<221> misc\_feature

<222> (21)..(120)

<223> at least one nt. of nt. 21-120 is present



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<223> nt. 124-223 are optionally present

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<210> 15
<211> 215
<212> DNA
<213> Artificial sequence

<220>
<223> Revers primer

<220>
<221> misc_feature
<222> (1)..(215)
<223> n is any nucleotide

<220>
<221> misc_feature
<222> (1)..(10)
<223> at least one nt. of nt. 1-10 is present

<220>
<221> misc_feature
<222> (16)..(115)
<223> at least one nt. of 16-115 is present

<220>
<221> misc_feature
<222> (116)..(215)
<223> nt. 116-215 are optionally present

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<210> 16
<211> 123
<212> DNA
<213> Artificial sequence

<220>
<223> Forward primer with 10-100 template specific sequence

<220>
<221> misc_feature

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<222> (24)..(123)  
 <223> n is any nucleotide

<220>  
 <221> misc\_feature  
 <222> (34)..(123)  
 <223> nt. 34-123 are each optionally present

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 nnnnnnnnnnn nnnnnnnnnnn nnnnnnnnnnn nnnnnnnnnnn nnnnnnnnnnn nnnnnnnnnnn 120  
 nnn 123

<210> 17  
 <211> 121  
 <212> DNA  
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<220>  
 <223> Reverse primer with 10-100 nt long template specific sequence

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 <221> misc\_feature  
 <222> (22)..(121)  
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<220>  
 <221> misc\_feature  
 <222> (32)..(121)  
 <223> nt. 32-121 are each optionally present

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 n 121

<210> 18  
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 <213> Artificial sequence

<220>  
 <223> Forward primer

<400> 18  
 ctagaaggga ggagaaaacc atg 23

<210> 19  
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<220>  
 <223> Reverse primer

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gatcaaaggc ggcctgcag g

21

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<220>  
 <223> Linker peptide

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<220>  
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 aatgcctttt gtgacggacg tcgcccgatg 150

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<220>  
 <223> Protogenitor template 12412

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<210> 23  
 <211> 150  
 <212> DNA  
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 <223> Protogenitor template 124-1d

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<210> 24  
 <211> 150  
 <212> DNA  
 <213> Artificial sequence

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 <223> Protogenitor template mycol

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 <212> DNA  
 <213> Artificial sequence

<220>  
 <223> Protogenitor template b3

<400> 25  
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<210> 26  
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<220>  
 <223> Protogenitor template b1

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<220>  
 <223> Protogenitor template 15112

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<210> 28  
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 <213> Artificial sequence

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<220>  
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<400> 29  
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<210> 30  
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 <212> DNA  
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<220>  
 <223> Derived nucleic acid building block sequence

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 gcttcgttcg acagtacaat cgatcaatta atggttcgag 100

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<220>  
 <223> Polynucleotide progenitor template 150am13\_00

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 gatgatcgtc ggcataga ccggcctgcc cggaatggat ctggtgatct tcccgaata 180  
 ttcgaccac ggcacatgt acgactccaa ggaaatgtac gataccgcgt ccgtcgtgcc 240  
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tccttatgag ttctacaaca aatggatcgc cgatccggaa ggcacccgcg aaatggtcga	960
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gcaccgtggc tacaccggca tgatcaattc cggcgagggc gaccgcggtg tcgcggcttg	900
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gatcagcctc atcatctgcg acgacggcaa ttaccccag atctggcgcg attgcgccat	540
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